Primary Evaluator: Date: 03-MAR-2006
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Registration Action Branch 1 (RAB 1)
Health Effects Division (HED) (7509C)

Approved by: Date: 03-MAR-2006
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This DER was originally prepared under contract by Dynamac Corporation (2275 Research Boulevard, Suite 300; Rockville, MD 20850; submitted 02/01/2006). The DER has been reviewed by the HED and revised to reflect current Office of Pesticide Programs (OPP) policies.

STUDY REPORT:

45710226 Shaw, D. (1997) Chlorothalonil Metabolism in the Lactating Goat: Lab Project Number: VCM 73/961389. Unpublished study prepared by Huntingdon Life Sciences Ltd. 143 p.

EXECUTIVE SUMMARY:

Vischim S.r.l has submitted a study investigating the metabolism of [phenyl-U-^{14}C]chlorothalonil (specific activity 19.8 mCi/mmol) in a lactating goat. The test substance was administered orally to a single goat at approximately 10 ppm in the diet; the dosing level was based on estimated feed consumption. The goat was dosed once daily for 5 consecutive days. Milk was collected twice daily throughout the study, and tissues (liver, kidney, muscle, and fat) were collected at sacrifice. The in-life phase and analytical phases of the study were conducted by Huntingdon Life Sciences Ltd. (Huntingdon, England).

Total radioactive residues (TRR) were 0.031-0.090 ppm in milk, 0.23 ppm in liver, 1.1 ppm in kidney, 0.02-0.03 ppm in fat, and 0.02 ppm in muscle. Radioactivity was highest in kidney and lowest in fat and muscle. Residues in milk did not appear to have reached a plateau by the end of the dosing period. A large portion of the administered dose was excreted, with urine, feces, and cage wash accounting for a total of ~51% of the administered dose. A total of 75.4% of the administered dose was recovered in goat matrices.

A large portion of the radioactivity (~52-82% TRR) was extracted from goat matrices using acetonitrile, acetonitrile/water, and/or acetone. The nonextractable residues of all matrices were subjected to enzyme hydrolyses, which released ~4-9% TRR in liver, fat, and muscle samples, and ~12-18% TRR in milk and kidney samples. Nonextractable residues were additionally subjected to mild acid and base hydrolyses, which released a total of ~6-17% TRR. Liver and kidney nonextractable residues were additionally subjected to acid hydrolysis at reflux, which released 16% and 6% TRR, respectively. Nonextractable residues accounted for <0.03 ppm (5.5-16% TRR) in milk, liver, fat, and muscle, and <6% TRR (0.12 ppm) in kidney. Residues were characterized primarily by high-performance liquid chromatography (HPLC) analysis, using thin-layer chromatography (TLC) analysis for confirmation of residue identity.

Approximately 4% TRR were identified in kidney, and 24-45% TRR were identified in milk, liver, fat, and muscle. Chlorothalonil was not detected in any goat matrix. Two metabolites were identified: 4-hydroxy chlorothalonil, at 41.1% TRR (0.025 ppm) in milk, 10.4% TRR (0.024 ppm) in liver, 4.1% TRR (0.045 ppm) in kidney, 18.2% TRR (0.005 ppm) in fat, and 12.8% TRR (0.002 ppm) in muscle; and 2,5,6-trichloro-1,3-dicyanobenzene, at 3.6% TRR (0.002 ppm) in milk, 13.1% TRR (0.030 ppm) in liver, 15.6% TRR (0.004 ppm) in fat, and 31.7% TRR (0.006 ppm) in muscle. Comparison of HPLC retention times indicated that the diglutathione conjugate of chlorothalonil was present in kidney extracts, at ~2% TRR. The remainder of the extractable radioactivity consisted of unknowns, each present at <0.08 ppm.

The petitioner stated that the enzyme, acid, and base hydrolysates of goat matrices were not analyzed because of low radioactivity levels. The hydrolysates from milk, liver, muscle, and fat each accounted for <0.05 ppm. The kidney hydrolysates contained greater amounts of radioactivity, at 17.5% TRR (0.19 ppm) in the enzyme hydrolysate, 6.9% TRR (0.076 ppm) in the 0.1 M HCl hydrolysate, 10.5% TRR (0.12 ppm) in the 0.1 M NaOH hydrolysate, and 6.1% TRR (0.067 ppm) in the 0.1 M HCl reflux hydrolysate. The petitioner should have attempted analysis of the radioactivity in the hydrolysates of kidney.

Samples were stored frozen prior to analysis, and most analyses were completed within ~7 months of collection. The petitioner provided data demonstrating that the metabolite profile was stable in milk and kidney during 7 months of frozen storage. In liver, muscle, and fat, decline in one major metabolite, 2,5,6-trichloro-1,3-dicyanobenzene, was observed during 7 months of storage, with a corresponding increase in the metabolite 4-hydroxy chlorothalonil. These data are adequate to support the study.

Based on the observed metabolites, chlorothalonil is metabolized in goats to 4-hydroxy chlorothalonil, 2,5,6-trichloro-1,3-dicyanobenzene, and the mono- and diglutathione conjugates of chlorothalonil (both conjugates were found in urine).

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the goat metabolism data are classified as scientifically unacceptable because radioactivity was insufficiently characterized in kidney. Because the submitted study was completed in 1997, it would be impractical for the petitioner to conduct additional work on kidney residues.

COMPLIANCE:

Signed and dated Good Laboratory Practice (GLP), Quality Assurance and Data Confidentiality statements were provided. No deviations from regulatory requirements were reported which would have an impact on the validity of the study.

A. BACKGROUND INFORMATION

Chlorothalonil is a broad spectrum, non-systemic protectant pesticide mainly used as a fungicide to control fungal foliar diseases of vegetable, field, and ornamental crops. It is also used as a wood protectant, antimold and antimildew agent, bactericide, microbiocide, algaecide, insecticide, and acaricide. The exact mechanism of action is not known.

TABLE A.1. Test Compound Nomenclature.

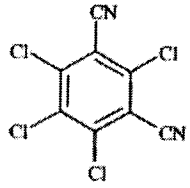
Compound	
Common name	Chlorothalonil
Company experimental name	N/A
IUPAC name	tetrachloroisophthalonitrile
CAS name	2,4,5,6-tetrachloro-1,3-benzenedicarbonitrile
CAS registry number	1897-45-6

TABLE A.2. Physicochemical Properties of the Technical Grade Test Compound: Chlorothalonil.

Parameter	Value	Reference
Melting point	250-251 °C	Chlorothalonil Reregistration Eligibility Decision, April 1999
Water solubility	0.6 ppm (25 °C)	Chlorothalonil Reregistration Eligibility Decision, April 1999
Solvent solubility	g/L at 25°C: acetone 20 dimethyl sulfoxide 20 cyclohexanone 30 dimethylformamide 30 kerosene <10 xylene 80	The Pesticide Manual, 8th ed.
Octanol/water partition coefficient, Log(K_{OW})	4.37×10^2	TOXNET database

B. EXPERIMENTAL DESIGN

B.1. Livestock

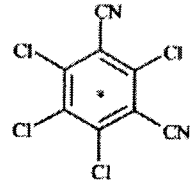
TABLE B.1.1. General Test Animal Information.

Species	Breed	Age	Weight at study initiation (kg)	Health Status	Description of housing/holding area
Lactating goat	British Saanen type	~3 years	47.5	The health of the goat was satisfactory at study initiation, and daily observations were made during the study period. No health issues were reported.	Stainless steel metabolism cage with steel mesh floor; lighting on a 12-hour light/12-hour dark cycle.

TABLE B.1.2. Test Animal Dietary Regime.				
Composition of Diet	Feed consumption (kg/day)	Water	Acclimation period	Predosing
Concentrate rations based on cereals and vegetable protein; nominally containing no antibiotics or other feed additives.	Not reported	Tap water, *ad libitum*	3 days	None

TABLE B.1.3. Test Animal Dosing Regime.				
Treatment Type	Treatment Level (ppm test material in vehicle)	Vehicle	Parameters	Timing/Duration
Oral	Approximately 10 ppm, based on assumed feed consumption of 2 kg/day.	Gelatin capsule	Capsule containing test substance administered via balling gun.	Once daily, after morning milking, for five consecutive days.

B.2. Test Materials

TABLE B.2.1. Test Material Characteristics.	
Chemical structure	
Radiolabel position	[phenyl-U-^{14}C]chlorothalonil
Lot No.	SPS/VCM/73/1
Purity	97.7-99.7% radiochemical purity
Specific activity	47.5 mCi/mmol (test substance) 19.8 mCi/mmol (dosing material)

The test substance was isotopically diluted with non-labeled chlorothalonil and mixed with acetone.

B.3. Sampling Information

TABLE B.3.1. Sample Collection Information.			
Milk collected	Urine, feces and cage wash collected	Interval from last dose to sacrifice	Tissues harvested and analyzed
600-1250 mL collected daily during the dosing period (average = 862 mL); 1110-1820 mL collected daily during the acclimation period (average = 1541 mL).	Urine, feces, and cage wash collected every 24 hours during dosing period up to sacrifice.	23 hours	Liver, kidneys, muscle (foreleg, loin, and rump), and fat (subcutaneous, omental, and perirenal).

Milk was collected twice daily: in the morning immediately prior to dosing, and in the afternoon.

B.4. Identification/Characterization of Residues

B.4.1. Sample Handling and Preparation

A subsample of each milk sample was radioassayed immediately after collection or stored refrigerated (4 °C) prior to radioassay. The remainder of each milk sample was frozen after collection and stored frozen (<-15 °C) until analysis. Tissue samples were stored frozen between collection and analysis.

To generate a milk sample for residue characterization/identification, portions of all milk samples were pooled in proportion to their total volumes. For fat and muscle, a composite sample was prepared using approximately equal amounts of the three types of tissue. All composite samples were radioassayed prior to extraction.

The procedures used to extract radioactivity from milk and tissue samples are presented below in Figures B.4.1 through B.4.5, which were copied without alteration from MRID 45710226. Because the initial extraction procedures are clearly delineated in Figures B.4.1 through B.4.5, they are not further discussed in this section.

Figure B.4.1. Extraction procedures for milk.

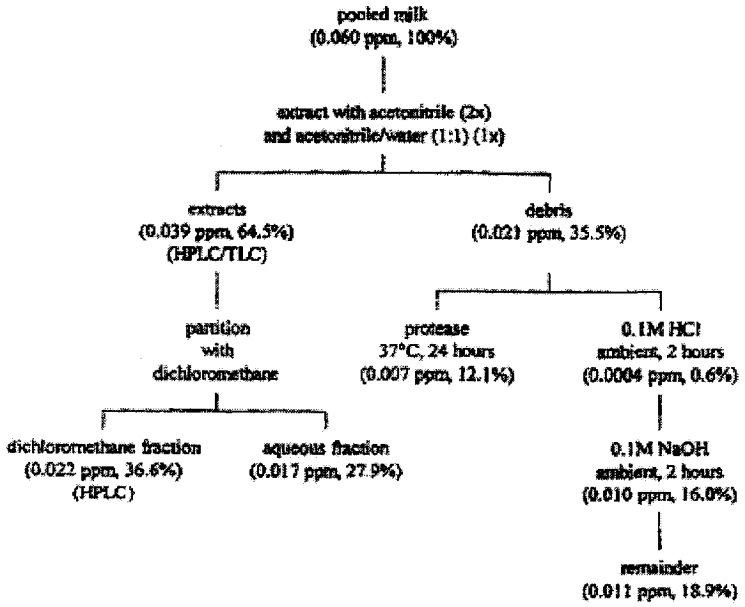


Figure B.4.2. Extraction procedures for liver.

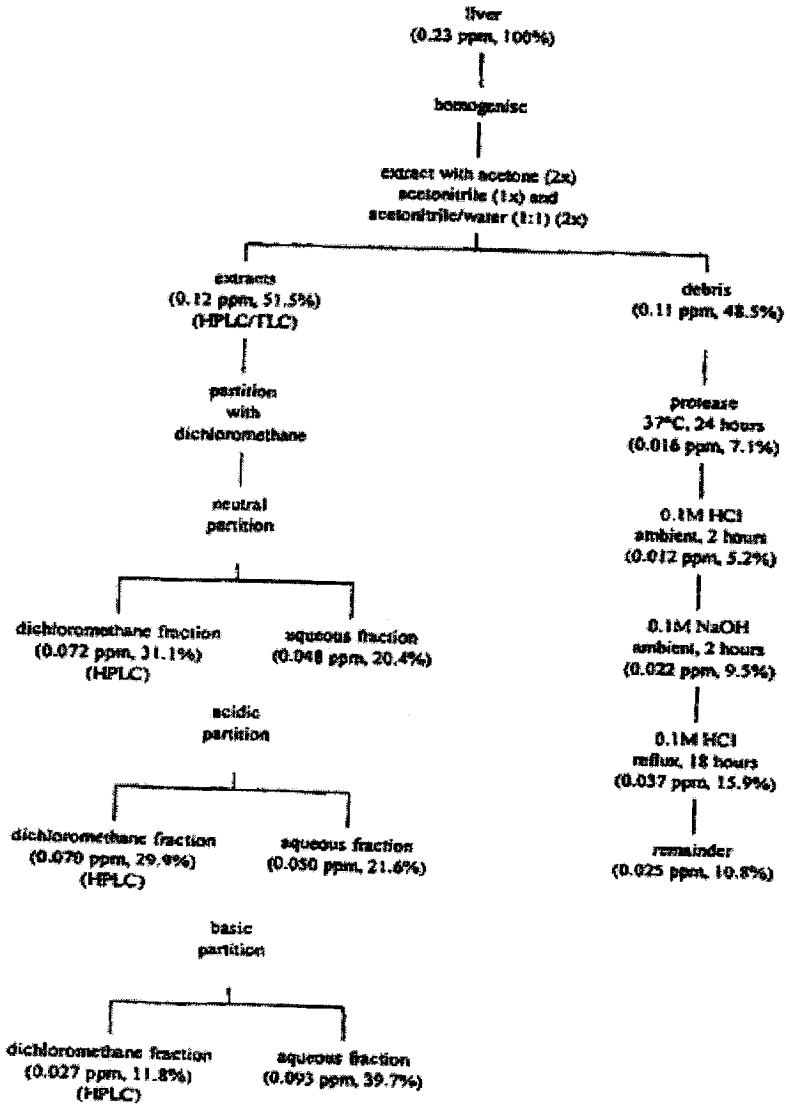


Figure B.4.3. Extraction procedures for kidney.

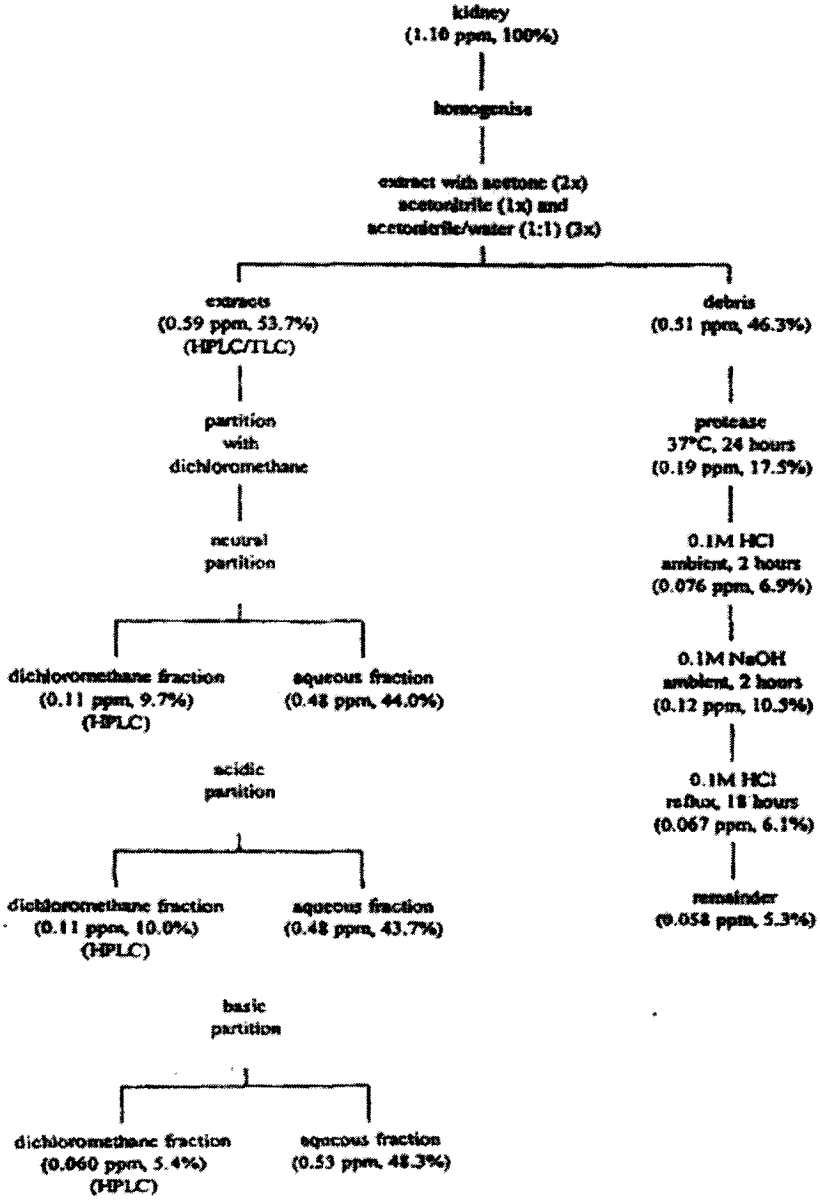


Figure B.4.4. Extraction procedures for fat.

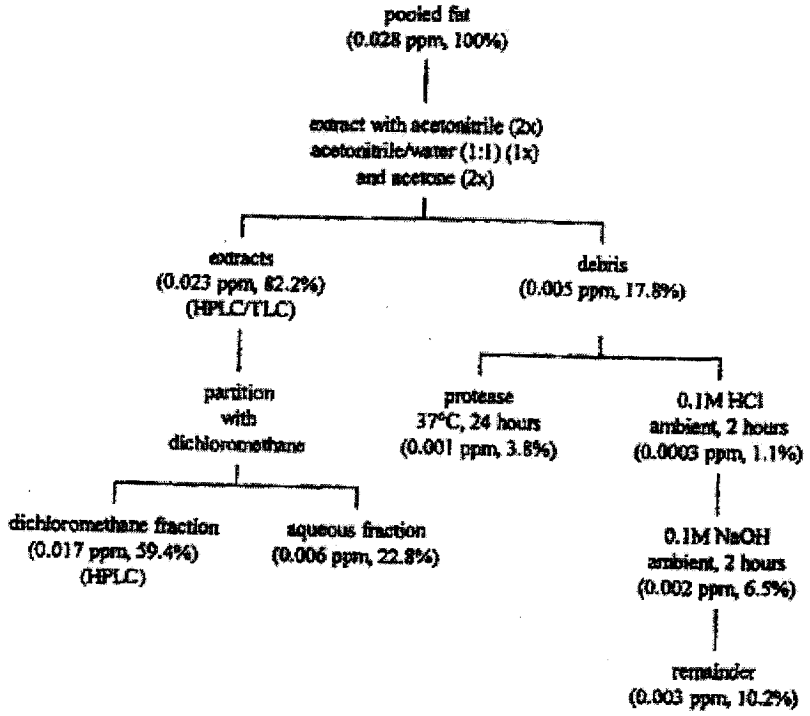
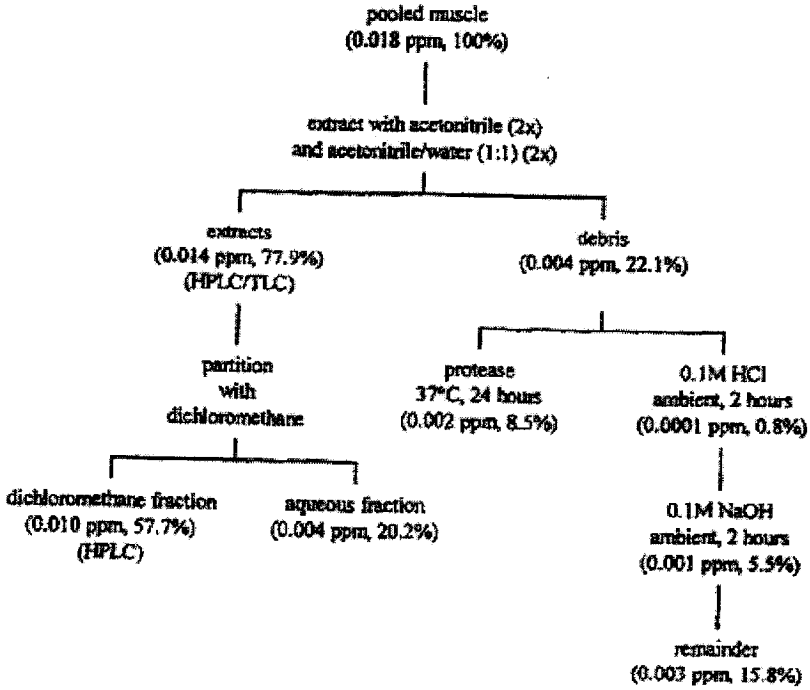


Figure B.4.5. Extraction procedures for muscle.



For the dichloromethane (DCM) partitioning steps, a second subsample of goat matrix was extracted as described in the figures, the combined extracts were concentrated to aqueous, and the extract was partitioned as described in the figures; for fat and muscle, the concentrated aqueous extracts were saturated with NaCl prior to DCM partitioning. For liver and kidney, the DCM partition was conducted on three separate subsamples of combined concentrated extracts: one at neutral pH; one after adjustment of extracts to pH 2 (using concentrated HCl); and one after adjustment of extracts to pH 12 (using 10 M NaOH).

For liver, a separate subsample was dialyzed in 0.01 M sodium phosphate buffer (pH 7.0, also containing 0.1% sodium dodecyl sulfate) to measure the proportion of radioactivity associated with covalently bound material. Aliquots of the dialysate were removed for radioassay after ~24, 72, and 168 hours; the buffer was replaced after ~24 and 72 hours. A subsample of kidney was subjected to the same procedures, removing aliquots of the dialysate after ~48, 96, and 168 hours.

B.4.2. Analytical Methodology

TRR were measured in milk, urine, and sample extracts and hydrolysates by liquid-scintillation counting (LSC). TRR in tissue and feces samples and extracted solids were determined by combustion/LSC. Kidney, muscle, and fat samples were treated with tissue solubilizer at 55 °C for up to 24 hours. When solubilization was complete, the samples were left in the dark for 24 hours and then radioassayed by LSC. TRR in solids remaining after enzyme and/or acid and base hydrolyses were calculated based on the amount of radioactivity in the solid prior to hydrolysis and the amount released by the hydrolysis procedures. No limits of detection or quantitation were reported.

Extracts were analyzed by HPLC and TLC. HPLC analyses were conducted on a system equipped with a phenyl column, a ultraviolet (UV) detector (254 nm), and a radioactivity detector, using a gradient mobile phase of 0.002 M 1-heptane sulfonic acid, 0.018 M sodium phosphate, and acetonitrile. Metabolites were identified by co-chromatography with non-labeled reference standards. The chemical names and structures of the reference standards used in this study are presented in Appendix I.

TLC analyses were conducted in the normal phase, using Kieselgel 60 F_{254} plates, or in the reversed phase, using C-18 plates. The following solvent systems were used: (1) hexane:DCM (1:1, v:v; normal phase); (2) methanol:water (9:1, v:v; reverse phase); (3) butan-1-ol:water:acetic acid (3:1:1, v:v:v; normal phase); (4) butan-1-ol:butan-2-one:water:ammonia solution (6:3:2:1, v:v:v:v; normal phase); and (5) acetonitrile:water (7:3, v:v; reverse phase). Radioactivity was detected and quantified using a bioimaging analyzer. Non-labeled standards were visualized by quenching the UV-fluorescent indicator on the plate. Metabolites were identified by co-chromatography with reference standards.

Liquid chromatography/mass spectroscopy (LC/MS) and LC/MS/MS analyses were conducted using a C-18 column, a gradient mobile phase of water and acetonitrile and MS and/or MS/MS detection. GC/MS analyses were conducted using a DB-5 column.

C. RESULTS AND DISCUSSION

The storage conditions and intervals for samples from the goat metabolism study are presented in Table C.1. Samples were stored frozen prior to analysis, and most analyses were completed within ~7 months of collection. The petitioner provided data demonstrating that the metabolite profile was stable in milk and kidney during 7 months of frozen storage. In liver, muscle, and fat, decline in one major metabolite, 2,5,6-trichloro-1,3-dicyanobenzene, was observed during 7 months of storage, with a corresponding increase in the metabolite 4-hydroxy chlorothalonil. These data are adequate to support the study.

TRR in goat milk and tissues are reported in Table C.2.1. TRR were 0.031-0.090 ppm in milk, 0.23 ppm in liver, 1.1 ppm in kidney, 0.02-0.03 ppm in fat, and 0.02 ppm in muscle from a goat dosed orally with [^{14}C]chlorothalonil at approximately 10 ppm in the diet for 5 consecutive days. Radioactivity was highest in kidney and lowest in fat and muscle. Residues in milk did not appear to have reached a plateau by the end of the dosing period. A large portion of the administered dose was excreted, with urine, feces, and cage wash accounting for a total of ~51% of the administered dose. A total of 75.4% of the administered dose was recovered in goat matrices.

The distribution of the radioactivity in goat matrices is presented in Table C.2.2. A large portion of the radioactivity (~52-82% TRR) was extracted from goat matrices using acetonitrile, acetonitrile/water, and/or acetone. The nonextractable residues of all matrices were subjected to enzyme hydrolyses, which released ~4-9% TRR in liver, fat, and muscle samples, and ~12-18% TRR in milk and kidney samples. Nonextractable residues were additionally subjected to mild acid and base hydrolyses, which released a total of ~6-17% TRR. Liver and kidney nonextractable residues were additionally subjected to acid hydrolysis at reflux, which released 16% and 6% TRR, respectively. Nonextractable residues accounted for <0.03 ppm (5.5-16% TRR) in milk, liver, fat, and muscle, and <6% TRR (0.12 ppm) in kidney. Residues were characterized primarily by HPLC analysis, using TLC analysis for confirmation of residue identity.

The characterization and identification of residues in goat matrices is summarized in Table C.2.3. Approximately 4% TRR were identified in kidney, and 24-45% TRR were identified in milk, liver, fat, and muscle. Chlorothalonil was not detected in any goat matrix. Two metabolites were identified: 4-hydroxy chlorothalonil, at 41.1% TRR (0.025 ppm) in milk, 10.4% TRR (0.024 ppm) in liver, 4.1% TRR (0.045 ppm) in kidney, 18.2% TRR (0.005 ppm) in fat, and 12.8% TRR (0.002 ppm) in muscle; and 2,5,6-trichloro-1,3-dicyanobenzene, at 3.6% TRR (0.002 ppm) in milk, 13.1% TRR (0.030 ppm) in liver, 15.6% TRR (0.004 ppm) in fat, and 31.7% TRR (0.006 ppm) in muscle. The remainder of the extractable radioactivity consisted of unknowns, each present at <0.08 ppm.

The identification of 4-hydroxy chlorothalonil in milk was confirmed by LC/MS/MS analyses. The major metabolite in liver was identified based on GC/MS analyses of the same metabolite isolated from feces. The GC/MS analyses indicated that the metabolite was a trichloro-1,3-

dicyanobenzene compound. Although the relative position of the chlorine atoms could not be determined, the petitioner concluded that the positions of the chlorine atoms would likely be the same as those of 4-hydroxy chlorothalonil; therefore, the metabolite was determined to be 2,5,6-trichloro-1,3-dicyanobenzene. The metabolite was identified in milk and tissues by HPLC co-chromatography.

The HPLC analyses of the initial extracts were used for residue characterization/identification. The petitioner additionally subjected the DCM and aqueous phases, obtained after partitioning the initial extracts with DCM, to HPLC analyses. These analyses showed that for milk, 4-hydroxy chlorothalonil was found in both phases, and less polar metabolites were only found in the DCM phase. For liver, partitioning at neutral and acidic pH yielded more radioactivity in the DCM phase than at basic pH; in all cases, the DCM phase consisted almost entirely of 4-hydroxy chlorothalonil and 2,5,6-trichloro-1,3-dicyanobenzene. For kidney, the majority of the radioactivity was found in the aqueous phase following partitioning at neutral, acidic, and basic pH; the metabolic profile in the DCM phase was similar at each pH. For fat, the majority of the radioactivity partitioned into the DCM phase; results from HPLC analysis of this phase were poor. For muscle, the majority of the radioactivity partitioned into the DCM phase; HPLC analysis revealed the same profile as that of the initial extract.

In kidney, the only metabolite identified was 4-hydroxy chlorothalonil; because this metabolite was only identified by HPLC co-chromatography, the petitioner considered it to be tentatively identified. One polar metabolite, comprising 13.5% TRR (0.15 ppm), was subjected to further analysis. One of the aqueous phases from DCM partitioning, which was found to contain the polar metabolite, was analyzed by TLC using polar solvent systems. These analyses indicated that the polar metabolite was comprised of a number of components, each ≤0.07 ppm. The fraction was subjected to acid hydrolysis (in 0.1 M HCl at 37 °C for 18 hours) and TLC analysis of the hydrolysate revealed additional components, confirming that the polar metabolite was multicomponent.

The metabolic profile in kidney was similar to that of urine; however, co-chromatography of kidney and urine extracts was inconclusive. Comparison of HPLC retention times indicated that the diglutathione conjugate of chlorothalonil was present in kidney extracts at ~2% TRR [the petitioner concluded that the monoglutathione conjugate of chlorothalonil was also present in kidney; however, based on the chromatograms included in the submission (Figure 14 of the submission), the study reviewer could not confirm that conclusion].

The petitioner stated that the enzyme, acid, and base hydrolysates of goat matrices were not analyzed because of low radioactivity levels. The hydrolysates from milk, liver, muscle, and fat each accounted for <0.05 ppm. The kidney hydrolysates contained greater amounts of radioactivity, at 17.5% TRR (0.19 ppm) in the enzyme hydrolysate, 6.9% TRR (0.076 ppm) in the 0.1 M HCl hydrolysate, 10.5% TRR (0.12 ppm) in the 0.1 M NaOH hydrolysate, and 6.1% TRR (0.067 ppm) in the 0.1 M HCl reflux hydrolysate. The petitioner should have attempted analysis of the radioactivity in the hydrolysates of kidney.

The results of the dialysis experiments with liver and kidney, in which the samples were subjected to dialysis with sodium phosphate buffer containing sodium dodecyl sulfate, were in good agreement with the values obtained for extractable radioactivity using the conventional extraction procedures.

C.1. Storage Stability

Sample storage conditions and intervals are presented in Table C.1. Samples were stored frozen prior to analysis. The petitioner reported the dates of initial analysis and the dates of the reanalyses that were conducted to support storage stability. Based on the MS chromatograms included in the submission, the LC/MS and GC/MS analyses were conducted up to 11 months after sample collection.

To provide supporting storage stability data, the petitioner reextracted samples of milk, liver, kidney, muscle, and fat approximately 6-7 months after the initial extraction. The extracts were analyzed (~1 month after extraction) and the results were compared with those from the initial analyses. For milk and kidney, the chromatographic profiles from the initial and final analyses were similar. For liver, the petitioner noted that the amount of the metabolite 2,5,6-trichloro-1,3-dicyanobenzene decreased in the stored sample, as compared with the initial analysis (from 13.1% TRR to 3.4% TRR), and that there was an increase in the amount of 4-hydroxy chlorothalonil (from 10.4% TRR to 25.3% TRR); this suggests that 2,5,6-trichloro-1,3-dicyanobenzene may be a precursor of 4-hydroxy chlorothalonil. In muscle and fat, similar significant increases in the levels of 4-hydroxy chlorothalonil were observed, with corresponding decreases in the levels of 2,5,6-trichloro-1,3-dicyanobenzene and other less polar metabolites.

The relative levels of metabolites that are reported in Tables C.2.2 and C.2.3 appear to be from the initial analyses.

HED concludes that the submitted storage stability data are adequate to support the goat metabolism study. Although some analyses may have been conducted outside the interval for which storage stability data were provided (~7 months), these analyses were conducted to identify isolated metabolites.

TABLE C.1. Summary of Storage Conditions.

Matrix		Storage Temperature (°C)	Actual Storage Duration (days)	Interval of Demonstrated Storage Stability (days)
Milk:	RAC	<-15	41-223	223
	Extract		10-31	--
Liver:	RAC		33-223	223
	Extract		18-31	--
Kidney:	RAC		33-223	223
	Extract		21-31	--
Fat:	RAC		55-223	223
	Extract		10-31	--
Muscle:	RAC		55-223	223

TABLE C.1. Summary of Storage Conditions.			
Matrix	Storage Temperature (°C)	Actual Storage Duration (days)	Interval of Demonstrated Storage Stability (days)
Extract		10-31	--

C.2. Identification, Characterization, and Distribution of Residues

TABLE C.2.1. TRR in Milk, Tissue and Excreta.			
Matrix	Collection Timing	% Administered Dose	ppm, [^{14}C]chlorothalonil equivalents
Urine	0-24 hours	8.2	--
	0-48 hours	9.1	--
	0-72 hours	9.9	--
	0-96 hours	10.7	--
	0-119 hours	11.6	--
Feces	0-24 hours	0.1	--
	0-48 hours	16.7	--
	0-72 hours	26.7	--
	0-96 hours	31.5	--
	0-119 hours	39.1	--
Cage wash	Study duration	0.3	--
Total excreted	Study duration	51.0	--
Milk	0-24 hours	0.3	0.031
	24-48 hours		0.051
	48-72 hours		0.068
	72-96 hours		0.080
	96-119 hours		0.090
Muscle, foreleg [1]	At sacrifice	0.02	0.02
Muscle, loin [1]	At sacrifice	0.02	0.02
Muscle, rump [1]	At sacrifice	0.02	0.02
Fat, omental	At sacrifice	0.08	0.03
Fat, perirenal	At sacrifice	0.02	0.03
Fat, subcutaneous	At sacrifice	0.02	0.02
Kidney	At sacrifice	0.2	1.1
Liver	At sacrifice	0.2	0.23
Bile	At sacrifice	0.03	1.3
Whole blood [1]	At sacrifice	0.06	0.31
GI tract	At sacrifice	0.44	--
GI tract contents	At sacrifice	23.0	--
Sum of Administered Dose (%)		75.4	

[1] Subsample.

TABLE C.2.2. Distribution of the Parent and the Metabolites in Goat Matrices when Dosed with Radiolabeled Chlorothalonil at Approximately 10 ppm in the Diet.[1]

Metabolite Fraction	Milk		Liver		Kidney		Fat		Muscle	
	TRR = 0.060 ppm		TRR = 0.23 ppm		TRR = 1.10 ppm		TRR = 0.028 ppm		TRR = 0.018 ppm	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
ACN/water (acetone)	64.5	0.039	51.5	0.12	53.7	0.59	82.2	0.023	77.9	0.014
Chlorothalonil	--	--	--	--	--	--	--	--	--	--
4-hydroxy chlorothalonil	41.1	0.025	10.4	0.024	4.1 [2]	0.045	18.2	0.005	12.8	0.002
2,5,6-trichloro-1,3-dicyanobenzene	3.6	0.002	13.1	0.030	--	--	15.6	0.004	31.7	0.006
Unknowns	13.5 [3]	0.008	22.2 [4]	0.051	46.2 [5]	0.51	37.0 [6]	0.010	24.7 [7]	0.004
Other	6.3	0.004	5.6	0.013	3.4	0.037	11.3	0.003	8.6	0.002
Unextractable residues	35.5	0.021	48.5	0.11	46.3	0.51	17.8	0.005	22.1	0.004
Protease hydrolysate	12.1 [8]	0.007	7.1	0.016	17.5	0.19	3.8 [8]	0.001	8.5 [8]	0.002
0.1 M HCl hydrolysate	0.6	0.0004	5.2	0.012	6.9	0.076	1.1	0.0003	0.8	0.0001
0.1 M NaOH hydrolysate	16.0	0.010	9.5	0.022	10.5	0.12	6.5	0.002	5.5	0.001
0.1 M HCl reflux hydrolysate			15.9	0.037	6.1	0.067				
Solids	18.9	0.011	10.8	0.025	5.3	0.058	10.2	0.003	15.8	0.003

[1] Shading indicates that the extraction step and/or characterization analysis was not conducted for the matrix in question.
[2] Tentative identification.
[3] A total of 4 unknowns, each ≤7.5% TRR (≤0.005 ppm).
[4] A total of 8 unknowns, each ≤7.6% TRR (≤0.017 ppm).
[5] A total of 15 unknowns, each present at ≤6.9% TRR (≤0.076 ppm), with the exception of one polar metabolite present at 13.5% TRR (0.15 ppm). TLC analysis of this metabolite indicated that it was multicomponent (each ≤0.07 ppm).
[6] A total of 4 unknowns, each ≤15.3% TRR (≤0.004 ppm).
[7] A total of 3 unknowns, each ≤18.3% TRR (≤0.003 ppm).
[8] For milk, fat, and muscle, protease hydrolysis was conducted on a separate subsample of nonextractable residues than that used for acid and base hydrolyses.

TABLE C.2.3. Summary of Characterization and Identification of Radioactive Residues in Goat Matrices when Dosed with Radiolabeled Chlorothalonil at Approximately 10 ppm in the Diet.

Compound	Milk		Liver		Kidney		Fat		Muscle	
	TRR = 0.060 ppm		TRR = 0.23 ppm		TRR = 1.10 ppm		TRR = 0.028 ppm		TRR = 0.018 ppm	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
Chlorothalonil	--	--	--	--	--	--	--	--	--	--
4-hydroxy chlorothalonil	41.1	0.025	10.4	0.024	4.1	0.045	18.2	0.005	12.8	0.002
2,5,6-trichloro-1,3-dicyanobenzene	3.6	0.002	13.1	0.030	--	--	15.6	0.004	31.7	0.006
Unknowns/other	19.8	0.012	27.8	0.064	49.6	0.55	48.3	0.014	33.3	0.006
Protease hydrolysate	--	--	7.1	0.016	17.5	0.19	--	--	--	--
0.1 M HCl hydrolysate	0.6	0.0004	5.2	0.012	6.9	0.076	1.1	0.0003	0.8	0.0001
0.1 M NaOH hydrolysate	16.0	0.010	9.5	0.022	10.5	0.12	6.5	0.002	5.5	0.001
0.1 M HCl reflux hydrolysate	--	--	15.9	0.037	6.1	0.067	--	--	--	--
Total identified	44.7	0.027	23.5	0.054	4.1	0.045	33.8	0.009	44.5	0.008

TABLE C.2.3. Summary of Characterization and Identification of Radioactive Residues in Goat Matrices when Dosed with Radiolabeled Chlorothalonil at Approximately 10 ppm in the Diet.

Compound	Milk		Liver		Kidney		Fat		Muscle	
	TRR = 0.060 ppm		TRR = 0.23 ppm		TRR = 1.10 ppm		TRR = 0.028 ppm		TRR = 0.018 ppm	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
Total characterized	36.4	0.022	65.5	0.151	90.6	1.00	55.9	0.016	39.6	0.007
Total extractable	81.1	0.049	89.2	0.205	94.7	1.04	89.8	0.025	84.2	0.015
Unextractable (PES)[1]	18.9	0.011	10.8	0.025	5.3	0.058	10.2	0.003	15.8	0.003
Accountability[2]	100		100		100		100		100	

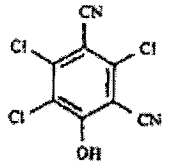
[1] Residues remaining after exhaustive extractions.
[2] Accountability = (Total extractable + Total unextractable)/(TRR from combustion analysis; see TABLE C.2.1) * 100.

C.3. Proposed Metabolic Profile

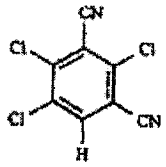
Based on the observed metabolites, chlorothalonil is metabolized in goats to 4-hydroxy chlorothalonil, 2,5,6-trichloro-1,3-dicyanobenzene, and the mono- and diglutathione conjugates of chlorothalonil (both conjugates were found in urine).

The figure below was copied without alteration from MRID 45710226.

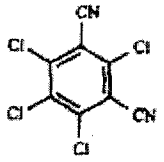
FIGURE C.3.1. Proposed Metabolic Profile of Chlorothalonil in Lactating Goat.



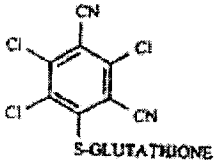
4-Hydroxy Chlorothalonil



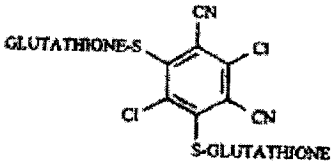
2,5,6-Trichloro-1,3-dicyanobenzene



Chlorothalonil



Monoglutathione conjugate



Diglutathione conjugate

TABLE C.3.1. Identification of Compounds from Metabolism Study.

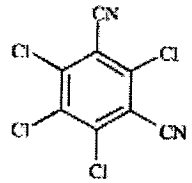
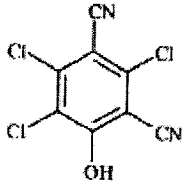
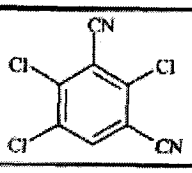
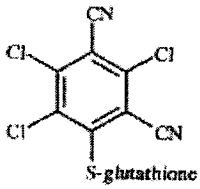
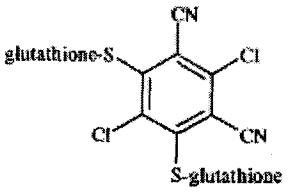
Common name/code Figure C.3.1 ID No.	Chemical name	Chemical structure
chlorothalonil	2,4,5,6-tetrachloro-1,3-benzenedicarbonitrile	
4-hydroxy chlorothalonil	4-hydroxy-2,5,6-trichloro-1,3-dicyanobenzene	

TABLE C.3.1. Identification of Compounds from Metabolism Study.

Common name/code Figure C.3.1 ID No.	Chemical name	Chemical structure
2,5,6-trichloro-1,3-dicyanobenzene	2,5,6-trichloro-1,3-dicyanobenzene	
monoglutathione conjugate of chlorothalonil [1]		
diglutathione conjugate of chlorothalonil [1]		

[1] Identified in goat urine.

D. CONCLUSION

TRR were 0.031-0.090 ppm in milk, 0.23 ppm in liver, 1.1 ppm in kidney, 0.02-0.03 ppm in fat, and 0.02 ppm in muscle from a goat dosed orally with [^{14}C]chlorothalonil at approximately 10 ppm in the diet for 5 consecutive days. Radioactivity was highest in kidney and lowest in fat and muscle. Residues in milk did not appear to have reached a plateau by the end of the dosing period. A large portion of the administered dose was excreted, with urine, feces, and cage wash accounting for a total of ~51% of the administered dose. A total of 75.4% of the administered dose was recovered in goat matrices.

A large portion of the radioactivity (~52-82% TRR) was extracted from goat matrices using acetonitrile, acetonitrile/water, and/or acetone. The nonextractable residues of all matrices were subjected to enzyme hydrolyses, which released ~4-9% TRR in liver, fat, and muscle samples, and ~12-18% TRR in milk and kidney samples. Nonextractable residues were additionally subjected to mild acid and base hydrolyses, which released a total of ~6-17% TRR. Liver and kidney nonextractable residues were additionally subjected to acid hydrolysis at reflux, which released 16% and 6% TRR, respectively. Nonextractable residues accounted for <0.03 ppm in milk, liver, fat, and muscle, and <6% TRR in kidney.

Approximately 41% TRR were identified in milk, and ~4-18% TRR were identified in liver, kidney, fat, and muscle. Chlorothalonil was not detected in any goat matrix. Two metabolites were identified: 4-hydroxy chlorothalonil, at 41% TRR in milk, 10% TRR in liver, 4% TRR in kidney, 18% TRR in fat, and 13% TRR in muscle; and 2,5,6-trichloro-1,3-dicyanobenzene, at

4% TRR in milk, 13% TRR in liver, 16% TRR in fat, and 32% TRR in muscle. Comparison of HPLC retention times indicated that the diglutathione conjugate of chlorothalonil was present in kidney extracts, at ~2% TRR. The remainder of the extractable radioactivity consisted of unknowns, each present at <0.08 ppm. Based on the observed metabolites, chlorothalonil is metabolized in goats to 4-hydroxy chlorothalonil, 2,5,6-trichloro-1,3-dicyanobenzene, and the mono- and diglutathione conjugates of chlorothalonil (both conjugates were found in urine).

E. REFERENCES

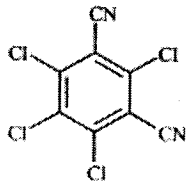
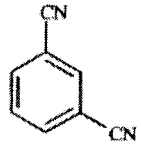
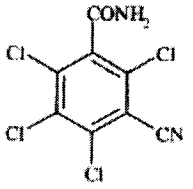
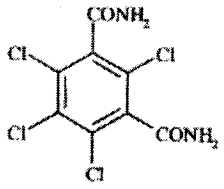
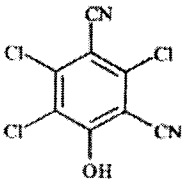
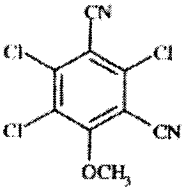
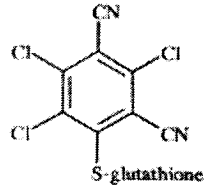
None.

F. DOCUMENT TRACKING

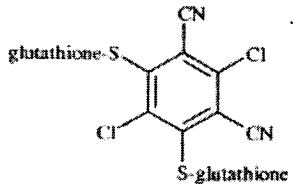
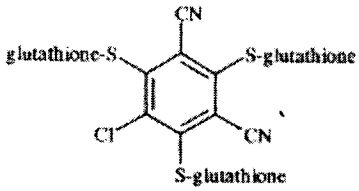
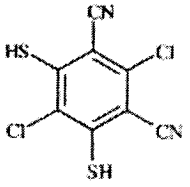
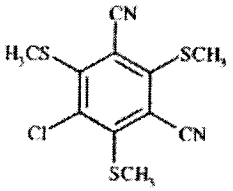
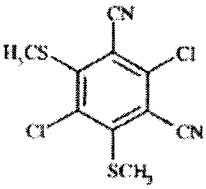
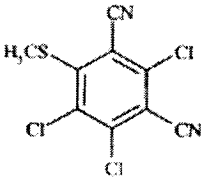
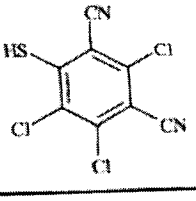
RDI: RAB1 Chemists (3/2/06)
DP#: 323612
PC Code: 081901

Template Version June 2005

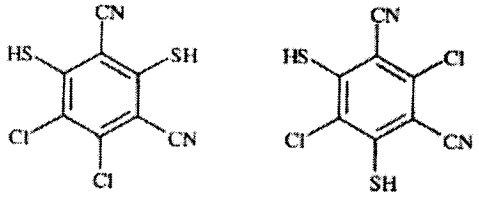
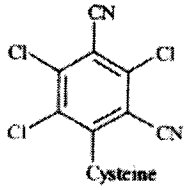
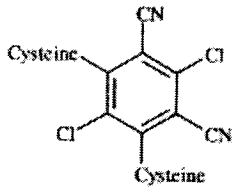
APPENDIX I. Chemical Names and Structures of Reference Standards Used in Chlorothalonil Goat Metabolism Study.

Common name; Company code	Chemical name	Chemical structure
chlorothalonil	2,4,5,6-tetrachloro-1,3-benzenedicarbonitrile	
isophthalonitrile		
monoamide of chlorothalonil	3-cyano-2,4,5,6-tetrachlorobenzamide	
diamide of chlorothalonil	2,4,5,6-tetrachloroisophthalamide	
4-hydroxy chlorothalonil	4-hydroxy-2,5,6-trichloro-1,3-dicyanobenzene	
4-methyoxy-2,5,6-trichloroisophthalonitrile	4-methyoxy-2,5,6-trichloroisophthalonitrile	
monoglutathione conjugate of chlorothalonil		

APPENDIX I. Chemical Names and Structures of Reference Standards Used in Chlorothalonil Goat Metabolism Study.

Common name; Company code	Chemical name	Chemical structure
diglutathione conjugate of chlorothalonil		
triglutathione conjugate of chlorothalonil		
2,5-dichloro-4,6-dithioisophthalonitrile	2,5-dichloro-4,6-dithioisophthalonitrile	
5-chloro-2,4,6-tri(methylthio)isophthalo-nitrile	5-chloro-2,4,6-tri(methylthio)isophthalonitrile	
2,5-dichloro-4,6-di(methylthio)isophthalo-nitrile	2,5-dichloro-4,6-di(methylthio)isophthalonitrile	
2,4,5-trichloro-6-(methylthio)isophthalonitrile	2,4,5-trichloro-6-(methylthio)isophthalonitrile	
2,4,5-trichloro-6-thioisophthalonitrile	2,4,5-trichloro-6-thioisophthalonitrile	

APPENDIX I. Chemical Names and Structures of Reference Standards Used in Chlorothalonil Goat Metabolism Study.

Common name; Company code	Chemical name	Chemical structure
4,5-dichloro-2,6-dithioisophthalonitrile; 2,5-dichloro-4,6-dithioisophthalonitrile [mixture]	4,5-dichloro-2,6-dithioisophthalonitrile; 2,5-dichloro-4,6-dithioisophthalonitrile	
monocysteine conjugate of chlorothalonil		
dicysteine conjugate of chlorothalonil		
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